SYNOPSIS

Ph.D in Food Service Management and Dietetics

STARCH CHARACTERIZATION, FUNCTIONAL PROPERTIES, PREBIOTIC POTENTIAL OF UNRIPE BANANA FLOURS AND DEVELOPMENT OF READY-TO-EAT AND READY-TO-COOK PRODUCTS

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1. Title

Starch Characterization, Functional Properties, Prebiotic Potential of Unripe Banana Flours and Development of Ready -to- Eat and Ready-to-Cook Products

2. Introduction

Consumers of today seek quick, quality food products that cater to diverse tastes and dietary preferences with health and wellness. Nevertheless, convenience attribute is also an important factor which decides consumer behaviour which has led to the popularity of RTE and RTC products.

Integrating attributes of health and wellness into RTE and RTC foods is inevitable in the modern day and is a promising solution for primary prevention of NCDs (non-communicable diseases). With increasing lifestyle-related disorders such as obesity and diabetes, there is a growing demand for convenience foods that offer balanced nutrition without compromising taste. The inclusion of functional ingredients, such as fiber, antioxidants, resistant starch, and plant-based proteins, can bring in health benefits of RTE and RTC products making them an appealing and mindful choice for a health-conscious population.

Bananas are wholesome, nutrient-rich fruits that serve as key sources of macronutrients, micronutrients, and phytonutrients (Afzal *et al*., 2022) with delicious taste and are available throughout. Bananas are highly preferred as they are versatile, affordable, and rich in resistant starch, which supports gut health. Although unripe bananas are often treated as common vegetables and consumed domestically in India, their potential as a significant prebiotic source remains largely untapped.

The resistant starch (up to 68% w/w) in unripe banana flour (UBF) is considered a useful ingredient in the food industry (Dibakoane *et al*., 2022). Resistant starch resists enzymatic digestion in the small intestine and reaches the colon intact, where it ferments, producing beneficial short-chain fatty acids such as acetate, propionate, and butyrate. This resistance to hydrolysis and digestion in the upper gastrointestinal tract slows glucose absorption, supporting glycemic regulation. Additionally, the resistant starch in banana flour promotes satiety. The prebiotic benefits contribute to reducing risks of NCDs including diabetes, obesity, cardiovascular disease, and colorectal cancer.

Musa paradisiaca cultivar Peyan (regional name) with an ABB genome, grown in Tamil Nadu and Kerala is known for its short, ridged fruits with a broad base and slightly tapered apex. The *Musa paradisiaca* cultivar Monthan (regional name), with an ABB genome, is a popular cooking banana in India. Its fruits are slightly curved, plump, irregularly five-sided with

prominent ridges, a broad base tapering to a long-angled pedicel, and a broad apex with a knob-shaped beak.

3. Definition of the problem

Despite the cultural and economic importance of *Musa* cultivars Peyan (ABB) and Monthan (ABB), there is a notable lack of research addressing their starch characteristics, nutritional and functional properties. Current literature primarily focuses on agricultural practices and propagation, as shown by a Google Scholar search yielding 608 results for 'Peyan,' with only a few studies directly investigating the food and nutritional value of 'Monthan'. Furthermore, research databases rarely provide details on the specific cultivar type and ripening stage, leading to a limited understanding of the nutritional variations among different banana cultivars and maturity levels. Considering that the properties of banana flours vary significantly by variety, comprehensive analysis of the starch characteristics, nutritional composition, and prebiotic potential of *Musa paradisiaca* cultivars Peyan and Monthan is essential to identify their potential for product development

Thus, the present study was executed with the following objectives.

4. Objectives

General Objectives

To

- Explore the suitability of unripe banana flour of *Musa paradisiaca* cultivars Peyan and Monthan as adjuncts for Ready -to- Eat (RTE) and Ready- to- Cook (RTC) products.
- Assess the Physicochemical properties, Prebiotic and Anti-oxidant Potential of unripe banana flours.
- Develop and evaluate unripe banana flours based Ready -to- Eat (RTE) and Ready -to- Cook (RTC) products.

Specific Objectives

- Assess Consumers' Knowledge, Attitude and Practice of RTE and RTC foods.
- Analyze the Starch characteristics, Functional properties, Nutrient & Phyto-nutrient composition of unripe banana flours.
- Study the Acceptability, Nutrient composition, and Shelf -life of the developed RTE and RTC products.
- Assess the Prebiotic potential, and Predicted GI (eGI) of the unripe banana flours and the developed RTE and RTC foods.

5. Methodology

Ethical approval was obtained for the study and the methodology was carried out in seven phases as follows

5.1. Phase 1: Exploring Consumers' Knowledge, Attitude and Practice of RTE and RTC Foods

5.2. Phase 2: Identification and Authentication of banana cultivars and Preparation of unripe banana flours

5.3. Phase 3: Determination of Starch Morphology, Characteristics and Functional Properties of unripe banana flours

5.4. Phase 4: Estimation of Nutrient Composition, Phyto nutrients, Anti -microbial activity and Anti- oxidant Potential of unripe banana flours

5.5. Phase 5: Development and Evaluation of RTE and RTC products incorporated with unripe banana flours

5.6. Phase 6 : Assessment of Prebiotic Potential of UBFs and selected RTE and RTC products

5.7. Phase 7 : Predicted GI and Consumer acceptability of selected RTE and RTC products

5.1.Phase 1: Exploring Consumers' Knowledge, Attitude, and Practice of RTE and RTC Foods

A baseline survey was carried out online during COVID-19 pandemic to elicit information on the Knowledge, Attitude, and Practice (KAP) regarding RTE and RTC foods. Adopting a convenience sampling method, 500 respondents aged 20 to 50 years were selected including young adults, middle-aged, and older adults. As adults, they may exhibit varying interest in functional RTE and RTC foods and typically make independent dietary and purchasing decisions, so they were selected with the following inclusion and exclusion criteria.

Inclusion Criteria:

- Respondents aged 20-50 years
- Both male and female
- Access to the internet and a valid email address

Exclusion Criteria:

- Respondents with limited digital literacy

A structured questionnaire encompassing demographic details, knowledge of functional foods, attitude towards functional ingredients in RTE and RTC foods, and consumption habits of

functional RTE/RTC foods was created using Google Forms. Cronbach's Alpha, a reliability measure, was used to check the internal consistency of the questions and a score of 0.7 was obtained, indicating acceptable reliability for the internal consistency of the questionnaire. A pilot test was conducted among 15 selected participants representing the respondents to identify any ambiguities in the questionnaire. Based on the feedback, minor revisions were implemented to improve clarity and effectiveness. The questionnaire was mailed to all 500 participants, and the percent response rate was 80% (400 respondents). The KAP score was calculated according to the FAO's KAP Manual (2014).

5.2. Phase 2: Identification and Authentication of banana cultivars and Preparation of unripe banana flours

The online survey results showed that only 29% of respondents considered unripe banana, sweet potato, and yam as functional food, driving home the fact that unripe banana health benefits have to be explored and made aware among the population. Hence the two cultivars of *Musa paradisiaca*, namely Peyan (ABB) and Monthan (ABB) were selected for the study.

5.2.1. Procurement and Authentication of Unripe bananas

The selected cultivars of *Musa paradisiaca*, Peyan (ABB) and Monthan (ABB), were procured from the wholesale fruit market in Chennai, Tamil Nadu. Their authenticity was verified by the Department of Pharmacognosy at the Siddha Central Research Institute, Chennai.

Plate 1: *Musa paradisiaca*

Peyan (ABB) **Monthan (ABB)**

5.2.2. Assessment of Physical characteristics

The physical attributes of 12 unripe banana fruits were determined using Dadzie and Orchard (1997) guidelines. Fruit length was measured from the distal end to the outer curve using a measuring tape. The circumference of each fruit was measured at its widest midpoint. The weight of the entire fruit, as well as the individual weights of the pulp and peel, were determined using a digital weighing balance (Ravi and Mustaffa,2013).

5.2.3. Determination and Validation of Ripening Stage

The ripening stages of the fruits were determined using the Von Loesecke (1950) scale, which categorizes the ripening process into seven stages based on the color of the peel. For this study, bananas at stage one of ripening were specifically chosen and the appropriate ripening stage was validated by the method described by Campuzano *et al*. (2018).

5.2.4. Preparation of unripe banana flour and flour yield

The method described by Kumar *et al*., (2019) was used for the preparation of unripe banana flour and calculating flour yield. The unripe bananas were washed under running tap water and peeled by hand using a sharp steel knife to minimise bruising damage. The peeled bananas were then sliced into one cm thick pieces. The slices were rinsed in a solution of citric acid (0.3g/100mL) to impede enzymatic browning and were dried in a tray drier at a temperature of 50ºC for 48 hours until they became brittle (break or shatter easily when subjected to stress, without significant deformation or bending). The dried slices were pulverized for 120 seconds and sieved through a 60-mesh sieve, and the resulting powder was collected and stored in HDPE bags at room temperature. Peyan banana flour (PUBF) and Monthan banana flour (MUBF) were used for further analysis.

The flour yield was standardized with three trials. The percentage yield of unripe banana flour (UBF) was calculated using the following formula.

$$\text{UBF yield (\%)} = \frac{(\text{Fresh weight of the pulp} - \text{Moisture content of the pulp}) \times 100}{\text{Fresh weight of the pulp}}$$

5.3. Phase 3: Determination of Starch Morphology, Characteristics and Functional Properties of unripe banana flours

5.3.1. Starch Morphology

Starch morphology and its characteristics help to understand the functional properties, digestibility, and potential applications of unripe banana flour and hence were studied.

Surface morphology observations of the unripe banana flour (UBF) were conducted using a scanning electron microscope (SEM) (FEI-Quanta FEG 200F) (Kumar *et al*., 2019).

Fourier-transform infrared (FT-IR) spectra were analysed using an FT-IR spectrophotometer (SHIMADZU IRAffinity-1S, Miracle 10 ZnSe Prism Plate) within the range of 600-3600 $cm^{-1.}$ (Kumar *et al*., 2021).

The X-ray diffraction (XRD) technique was employed to examine the crystallinity of the unripe banana flour. X-ray diffraction (XRD) patterns were generated using an X-Pert Pro PANalytical instrument. The relative crystallinity (%) was determined from the diffractograms using OriginPro 2023b Software. (Campuzano *et al.*,2018).

RC (%) = Crystalline peaks / (Crystalline peaks + Amorphous peak) * 100

5.3.2. Determination of Total Starch, Resistant Starch, Amylose, Amylopectin

Total starch was estimated by enzymatic method outlined by Goni *et al*., (1997). Resistant and Digestible Starch was determined by AOAC 2002.02 method. Amylose was estimated by the method of McCready *et al*. as outlined by Sadasivam and Manickam, 2008. The difference between the total starch content and the determined amylose content represents the amylopectin content.

5.3.3. Determination of Thermal Properties- Differential Scanning Calorimeter (DSC) Analysis

The thermal properties of the samples were assessed using a differential scanning calorimeter (NETZSCH DSC 204 F1 PHOENIX). The thermograms of the samples were generated with NETZSCH Proteus software. (Kumar *et al*., 2021).

5.3.4. Determination of Physicochemical properties

The analysis of physicochemical properties namely bulk density, oil absorption capacity, foaming capacity and emulsion activity and stability were determined by Wani *et al.,* (2010) method.

5.3.5. Determination of flour hydration properties

Flour hydration properties namely water solubility index (WSI), water absorption capacity (WAC), water absorption index (WAI), water holding capacity (WHC), and swelling power (SP) were determined by Cornejo and Rosell (2015) method. The swelling volume was determined as described by Buckman *et al*., (2017).

5.3.6. Determination of Flour gelling characteristics

Flour gelling properties namely least gelation capacity, light transmittance and syneresis were assessed. Least gelation capacity was determined using the method outlined by Chandra *et al* (2015). The light transmittance was measured every 24 hours for 5 days by determining the absorbance at 640 nm using a UV spectrophotometer, with a water blank as reference (Wani *et al*., 2010). For measuring syneresis Wani *et al*., (2010) method was used.

5.4. Phase 4: Estimation of Nutrient Composition, Phytonutrients, Anti -microbial activity and Anti- oxidant Potential of unripe banana flours

5.4.1. Nutrient composition

The unripe banana flours from Peyan (PUBF) and Monthan (MUBF) cultivars were evaluated for their moisture (925.10: AOAC, 2005), ash (923.03: AOAC, 2005), TSS and pH (942.15 and 981.12: AOAC, 2005). Total Soluble Solids (TSS) was determined using hand held Atago Refractometer (Salvador *et al*., 2007).

Protein content was determined using the Kjeldahl method with a conversion factor of Nx6.25 (AOAC, 2005), and fat content was analyzed following AOAC method 960.39 (2005). Total carbohydrates were assessed using the Anthrone method as outlined by Sadasivam and Manickam (2008). Total sugars were measured by the Nelson-Somogyi method (Nelson, 1994), and reducing sugars were quantified using the phenol-sulfuric acid assay (Sadasivam and Manickam, 2008). Total dietary fiber was evaluated using the Sigma TDF 100A kit based on AOAC 16th edition (1997), with crude fiber and cellulose determined according to Sadasivam and Manickam (2008). All the analysis were carried out on dry weight basis.

Vitamin B6 was quantified using the HPLC method outlined in EN 15652:2009 (FSSAI). Vitamin C content was determined following the method described by Silva *et al*. (1999). Mineral analysis for calcium, phosphorus, and iron was performed according to Ranganna (1997). Potassium content was analyzed using the AOAC method 999.19 via ICP-OES.

5.4.2. Estimation of Phytonutrients

Preliminary qualitative screening for phytochemicals -Alkaloids, Flavonoids, Sterols, Terpenoids, Anthraquinone, Anthocyanin, Phenolic compounds, Quinones, Tannin, Saponin, Cardiac glycosides, Glycosides, Lignin, Coumarins, Volatile oils was performed using standard methods (Sorescu *et al*., 2018).

Total phenolic content was measured using the Folin-Ciocalteu assay with gallic acid as the standard (Singleton *et al*., 1977). Total tannins were determined by Folin-Ciocalteu method, using tannic acid as the standard (Sadasivam and Manickam, 2008). Flavonoid content was assessed by the aluminium chloride colorimetric method, with quercetin as the standard (Alothman *et al*., 2009). Phytic acid was quantified using sodium phytate as the standard based on the method by Davies and Reid (1979).

5.4.3. Anti -microbial activity

The agar well diffusion method was used to evaluate the antibacterial activity of the UBFs extracts on *E. coli* (MTCC 452) and *Staphylococcus aureus* (MTCC 096) (Balouiri *et al*., 2016).

5.4.4. Anti -oxidant assays

Anti-oxidant potential was determined by three methods. DPPH free radical scavenging activity was assessed according to the method described by Carciochi *et al*. (2014). The ABTS free radical scavenging activity assay was performed following the protocol outlined by Re *et al*. (1999). Additionally, the Ferric Reducing Antioxidant Power (FRAP) assay was conducted based on the methodology established by Benzie and Strain (1996).

5.5. Phase 5 : Development and Evaluation of RTE and RTC products incorporated with unripe banana flours

In line with the objective of developing functional RTE and RTC foods, this study focused on incorporating unripe banana flour as a functional ingredient to enhance nutritional and phytonutrient content, increase resistant starch and fiber, and provide antioxidant and prebiotic benefits. Additionally, findings from the Phase 1 consumer survey highlight strong consumer interest in incorporating functional ingredients into RTC and RTE products, further supporting this approach.

Two product categories, baked and extruded, were selected for developing RTE and RTC foods. A total of number of five products namely jeera (cumin) cookies, muffins, and baked choco cereals were chosen for RTE category and noodles and pasta were chosen for the RTC category. The RTE recipes were prepared using the standard recipe making from the cook books and the RTC products were adapted from research papers (Vernaza *et al*. 2011, Ovando-Martinez *et al*. 2008).

All the five products were developed by replacing wheat flour with PUBF and MUBF in the variations of 15%, 30% and 50% level and the products were tried out and standardized. These products were treated as the test products. The control product was developed with 100% of wheat flour alone for all the developed five products for comparison purposes.

The sensory acceptability study was carried out for the 21 products (jeera cookies, muffins, baked choco cereals with PUBF and MUBF and three Control) formulated in RTE category and 14 products (noodles and pasta with PUBF and MUBF and two control) formulated in RTC category. Fifteen semi trained panel members evaluated the sensory attributes of the products on a 9-point Hedonic scale (Lim, 2011)

Among the three variations, 30% PUBF and 30% MUBF incorporated products were shortlisted for further evaluation based on their high acceptability scores. The physical properties of RTE and cooking properties of RTC was assessed. The nutrient profile and resistant starch of

the products were analyzed using standard protocols. For cookies and muffins, the parameters - weight, bulk density, diameter, and thickness was determined using standard methods (Bala *et al*., 2015). Cooking time for noodles and pasta was determined according to the method described by Yadav *et al*. (2014). Cooked weight was measured following the procedure outlined by Galvez and Resurreccion (1992), while cooking loss was assessed according to AACC (2000).

A comprehensive analysis of nutritional parameters was conducted, including moisture content (AOAC, 2005, method 925.10), total minerals (AOAC, 2005, method 923.03), protein content determined by the Kjeldahl method with a conversion factor of Nx6.25 (AOAC, 2005, method 978.04), and fat content analyzed using method 960.39 (AOAC, 2005). Total carbohydrates were assessed using the anthrone method as outlined by Sadasivam and Manickam (2008), while total sugars were measured by the Nelson-Somogyi method (Nelson, 1994). Total dietary fiber was quantified using the Sigma TDF 100A kit based on AOAC 16th edition (1997). Resistant starch was determined for both uncooked and cooked noodles and pasta to evaluate the degradation of resistant starch during the moist cooking.

Real time shelf-life analysis was performed to ensure the quality of the developed products over a period of 0-5 days for cookies and muffins and 0-90 days for baked choco cereals, noodles, and pasta. Peroxide value was assessed according to the AOAC-965.33 method. Total plate count was determined based on FSSAI guidelines (2016).

Product formulations with PUBF incorporated at a level of 30% in Jeera cookies (RTE category) and Noodles (RTC category) were shortlisted based on sensory acceptability, high resistant starch, and dietary fiber content for further evaluation of prebiotic potential and predicted GI.

5.6. Phase 6: Assessment of Prebiotic Potential of UBFs and selected RTE and RTC products

Lactobacillus acidophilus (ATCC 314), *Bifidobacterium longum* (ATCC BAA 999), and *Lactobacillus paracasei* (ATCC BAA 2839) were selected to assess growth promotion by UBFs representing the common gut microflora. Inulin, the established prebiotic was used as reference standard and MRS media with one percent dextrose was used as control carbohydrate. Changes in pH, optical density, and colony count were tracked over 24-96 hours (Powthong *et al*., 2020).

Prebiotic index expresses a quantitative value for comparison of different prebiotic carbohydrates. When the ratio expressed in the equation below surpasses one, it signifies that the microorganism's growth is stimulated by the tested prebiotic in contrast to the control carbohydrate (Palframan *et al*., 2003).

The prebiotic index (PI or I_{preb}) was calculated to determine the effectiveness of UBF as a prebiotic.

$$I_{preb} = \frac{CFU\ of\ probiotics\ in\ prebiotic\ carbohydrate}{CFU\ of\ probiotics\ in\ control\ carbohydrate}$$

The antimicrobial activity of fermentation supernatant at 96 h was assessed using the agar well diffusion method (Balouiri *et al*., 2016) against *E. coli* MTCC 452.

5.7. Phase 7: Predicted GI and Consumer acceptability of selected RTE and RTC products

In vitro starch digestion and predicted glycemic index was determined by Goni *et al*. (1997) method. The product acceptance and purchase intent were assessed by 50 participants from general population selected based on convenience sampling. A 5- point purchase intent scale used by Mora *et al*. (2022) was adapted for the study.

6. Results

The salient findings of the study carried out in seven phases are presented as follows.

6.1. Salient Findings of Phase 1

- Seventy-six percent of the participants were aware of functional foods/nutraceuticals. Twenty nine percent considered unripe banana, sweet potato, and yam as functional foods.
- Dietary fiber-rich foods had the highest preference rate (52%).
- A significant correlation was found between education and knowledge and attitude scores of the respondents. Consumption pattern and age, education, or income did not show significant relation.

6.2. Salient Findings of Phase 2

- The moisture content of fresh unripe bananas (*Musa paradisiaca* cv. Peyan and Monthan) was 69.56 ± 1.48% and 76.80 ± 2.49%, respectively.
- The total soluble solids (TSS) of the fresh fruit ranged from 1.13 ± 0.11 to 1.20 ± 0.20° Brix, which is low due to the bananas being in the first stage of ripening.
- Monthan fruit exhibited significantly greater fruit length and circumference and the average fruit weight was 74.42 ±4.15 g for Peyan and 192.71±4.81g for Monthan. Peyan had thicker peel and recorded lower pulp and peel ratio of 1.70±0.24. The flour yield for Peyan was 30 %, whereas for Monthan, it was 23 %.

6.3. Salient Findings of Phase 3

- *Musa paradisiaca* Peyan flour (PUBF) exhibited a combination of irregularly shaped round, oval, spherical, and occasionally flattened or elongated granules. In contrast, *Musa paradisiaca* Monthan flour (MUBF) predominantly showed elongated, oval-shaped granules with a few spheroid forms. The starch granules appeared intact, without any fractures.
- Based on the diffraction patterns depicted both PUBF and MUBF possess type B crystallinity. XRD analysis indicated that PUBF had a relative crystallinity percentage of 8.91%, while MUBF exhibited 13.06%.
- In FTIR spectra analysis the band observed at 3300 cm^{-1} in the infrared spectrum of unripe banana flours indicates the stretching vibrations of O–H bonds, signifying the presence of hydroxyl groups. Additionally, the band at 1627-1635 cm^{-1} signifies C=C stretching. Both cultivars displayed a peak at 1002 cm^{-1}, attributed to the stretching of carbonyl bonds.
- The total starch content of PUBF and MUBF was found to be 86.47 ± 3.05% and 82.94 ± 3.05%, respectively. Both varieties exhibited 23% amylose, 44% resistant starch and 43% digestible starch.
- The gelatinization temperature of banana flours ranged from 85.6 to 88.3 °C
- The bulk density of unripe banana flour in the current study was recorded as 0.68 - 0.69 g/ cm3. The emulsion stability test resulted in a negative result. No significant difference was observed between oil absorption capacity of PUBF and MUBF.
- A linear increasing trend in Water Absorption Index (WAI) was observed from 50ºC to 90ºC for MUBF whereas PUBF had the peak hydration and gelling capacities at 70ºC. The swelling power and the swelling volume of MUBF had a higher value.
- Partial gelation was observed at a starch concentration of 10%, while complete gelation occurred at a concentration of 12%. Storing UBF samples at 4 ºC for up to 120 hours, resulted in a decreasing trend in light transmittance and increased syneresis.

6.4. Salient Findings of Phase 4

- The Unripe banana flours exhibited a total carbohydrate content of 86.7 - 88 g/100g, protein 4.89-6.15g/100g, fat 1.36-1.96g/100g, dietary fiber 13.4 – 13.77g/100g. PUBF and MUBF were rich in potassium and the values ranged from 1390 to 1403 mg/100g.
- Qualitative screening for phytochemicals revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, and volatile oils. PUBF exhibited a significantly higher total

phenolic content of 118.85±4.19 mg GAE/100g compared to Monthan (87.15 ±6.17 mg GAE/100g). The tannins and phytic acid in PUBF were higher compared to MUBF. MUBF exhibited a significantly higher flavonoid (178.89±6.73 mg QE/100g) compared to PUBF (112.78±13.47 mg QE/100g).

- Methanolic extracts of PUBF exhibited 13 mm zone of inhibition against *E. coli* a gram-negative bacterium while MUBF showed 11 mm inhibition.
- Both UBF extracts showed 60-62 % inhibition in DPPH and ABTS assay. In FRAP assay PUBF exhibited a higher total anti-oxidant capacity (16.75 ± 0.48 mg AAE/g) when compared to MUBF (13.64 ± 0.64 mg AAE/g).

6.5. **Salient Findings of Phase 5**

- For the five products developed, the products with variation of 30% PUBF and MUBF incorporation demonstrated high acceptability scores.
- The total dietary fiber content of PUBF and MUBF cookies was higher than control. Resistant starch (RS) was significantly high for PUBF incorporated cookies. Protein levels in the UBF muffins ranged from 5.82 to 5.94 g/100 g. Dietary fiber and resistant starch levels in the UBF muffins were 4 – 4.5 g/100 g whereas the control exhibited a lower dietary fiber content of 3 g/100 g and resistant starch of 2.6 g/100g. The dietary fiber (10g/100g) and RS (6g/100g) in PUBF baked choco cereals were significantly higher than MUBF and control.
- In RTC category, PUBF and MUBF noodles had a protein content of 4.9- 5.34 g/100g. The dietary fiber content of 30% PUBF noodles was 7 g/100g whereas MUBF and control had 6 g/100g. In dry UBF noodles, the resistant starch (RS) content was 36 g/100 g, which decreased to 9 g/100 g in the cooked noodles but significantly higher than the control (2 g/100 g). PUBF pasta showed a notable fiber content of 6.5 g/100 g. Cooked UBF pasta also retained a higher RS content (11-12 g/100 g) compared to the control (3.7 g/100 g).
- Peroxide values of all the products were within the acceptable range (20 meq peroxide/kg) as proposed by CODEX/FAO standards. The microbial count of the developed products was within the standard ($<10^4$ cfu/g).

6.6. Salient Findings of Phase 6

- The fermentation of unripe banana flour by the selected probiotics *(Lactobacillus acidophilus, Bifidobacterium longum and Lactobacillus paracasei)* resulted in a decrease in the pH of the growth medium from 6 to 4. The reduction in pH can be linked to the

prebiotic activity of the unripe banana flour. The optical density (OD) of UBFs incorporated media with each of the probiotics showed an increase in OD.

- The number of colony formation units of *L. acidophilus* increased from 69 x 10^5 to 43 x 10^7 and from 66 x 10^5 to 38 x 10^7 in media with PUBF and MUBF respectively from 24 hours to 96 hours. Similar trend was observed for *Bifidobacterium longum* and *L. paracasei.*
- The prebiotic index (PI) values over 96 hours indicate positive growth effects for all tested carbohydrates, with all PI values above one. *Lactobacillus acidophilus* had the highest mean PI with Peyan flour (1.40).
- The fermentation supernatant of PUBF with *L. acidophilus* showed a 13 mm inhibition zone, indicating strong antibacterial properties, while MUBF and inulin had 11 mm. For *L. paracasei*, PUBF and MUBF had a 12 mm inhibition zone, slightly higher than inulin (11 mm).
- Among the selected two products with 30% PUBF, the Peyan cookies showed a higher prebiotic index for *L. acidophilus* at 72 hours (1.45). Peyan noodles exhibited higher PI values for *L. acidophilus* 1.50 at 96 h, which was the highest recorded PI across all samples.

6.7. Salient Findings of Phase 7

- The PUBF cookies and noodles exhibited a medium estimated glycemic index (eGI) of 62.7 and 63.2 respectively. Comparatively the control products were in high eGI category.
- The consumer purchase intent survey revealed that 76% of participants expressed they would "probably purchase" PUBF cookies, while PUBF noodles received 84% in the same category.

7. Conclusion

Unripe banana flour derived from *Musa paradisiaca* cultivars Peyan and Monthan demonstrates significant potential as a functional ingredient for ready-to-eat (RTE) and ready-to-cook (RTC) food products. The analysis revealed structural characteristics of the flour are suitable for food applications. The favorable physicochemical properties, flour hydration and gelling properties, appreciable phytonutrients and anti- oxidant capacity, substantial resistant starch and dietary fiber levels place it as a viable and potential adjunct in RTE and RTC foods. The study findings implies that the incorporation of *Musa paradisiaca* cultivars Peyan and Monthan unripe

banana flours into food formulations can enhance health benefits, thus catering to the growing demand for nutritious, convenient food options.

Recommendations

1. Explore applications of unripe banana flours in a wider range of products.
2. Conduct of in-vivo studies to investigate the prebiotic potential of unripe banana flours.

Limitations

1. Due to COVID-19 restrictions during the study period, only an online baseline survey was conducted, limiting participant diversity and response rates.
2. The pandemic extended the timeline for laboratory analyses, resulting in a delay that prevented the execution of in vivo studies for glycemic index.

8. References

1. Afzal, M. F., Khalid, W., Akram, S., Khalid, M. A., Zubair, M., Kauser, S., Mohamed Ahmed, K. A., Aziz, A., & Siddiqui, S. A. (2022). Bioactive profile and functional food applications of banana in food sectors and health: a review. *International Journal of Food Properties*, *25*(1), 2286–2300. https://doi.org/10.1080/10942912.2022.2130940
2. Alothman, M., Bhat, R., & Karim, A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, *115*(3), 785–788. https://doi.org/10.1016/j.foodchem.2008.12.005
3. American Association of Cereal Chemists (AACC). (2000). Approved methods of the Association of Cereal Chemist (10th ed.). St.Paul, MN: American Association of Cereal Chemists, University of Michigan
4. Bala, A., Gul, K., & Riar, C. S. (2015). Functional and sensory properties of cookies prepared from wheat flour supplemented with cassava and water chestnut flours. *Cogent Food & Agriculture*, 1(1), 1019815. https://doi.org/10.1080/23311932.2015.1019815
5. Balouiri, M., Sadiki, M., & Koraichi, S. I. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005
6. Benzie, I. F., & Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as measurement of ―antioxidant power- The FRAP assay. *Anal Bio chem*, 239, 70-76.
7. Buckman, E. S., Oduro, I., Plahar, W. A., & Tortoe, C. (2017). Determination of the chemical and functional properties of yam bean (*Pachyrhizus erosus* (L.) Urban) flour for food systems. *Food Science & Nutrition*, 6(2): 457–463. https://doi.org/10.1002/fsn3.574
8. Campuzano, A., Rosell, C. M., & Cornejo, F. (2018). Physicochemical and nutritional characteristics of banana flour during ripening. *Food Chemistry*, *256*, 11–17. https://doi.org/10.1016/j.foodchem.2018.02.113
9. Carciochi. R., Manrique, G., & Dimitrov, K. (2014). Changes in phenolic composition and antioxidant activity during germination of quinoa seeds (*Chenopodium quinoa* Willd*.). Int Food Res*, 21 (2):767-773.

10. Chandra, S., Singh, S and Kumari, D. (2015). Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology*, 52: 3681–3688. https://doi.org/10.1007/s13197-014-1427-2
11. Cornejo, F and Rosell, C. M. (2015). Physicochemical properties of long rice grain varieties in relation to gluten free bread quality. *LWT*, 62(2): 1203–1210. https://doi.org/10.1016/j.lwt.2015.01.050
12. Dadzie, B.K., and Orchard, J.E. (1997). Routine Post-Harvest Screening of Banana/Plantain Hybrids: Criteria and Methods. INIBAP Technical Guidelines 2, International Plant Genetic Resources Institute, Montpellier, pp 75-85.
13. Davies, N.T., Reid, H. (1979). An evaluation of phytate, zinc, copper, iron and manganese content and availability from soyabean textured vegetable protein meat substitutes or meat extruders. *Br J Nutr,* 41:579.
14. Dibakoane, S. R., Du Plessis, B., Da Silva, L. S., Anyasi, T. A., Emmambux, M. N., Mlambo, V., & Wokadala, O. C. (2022). Nutraceutical Properties of unripe banana flour Resistant Starch: a review. *Starch - Stärke*, *75*(9–10). https://doi.org/10.1002/star.202200041
15. Galvez, F. C. F., & Resurreccion, A. (1992). Reliability of the Focus Group Technique in determining the Quality Characteristics of Mungbean [*Vigna Radiata (L.) Wilczec*] Noodles. *Journal of Sensory Studies*, *7*(4), 315–326. https://doi.org/10.1111/j.1745-459x.1992.tb00197.x
16. Goñi, I., García-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, *17*(3), 427–437. https://doi.org/10.1016/s0271-5317(97)00010-9
17. Kumar, P. S., Saravanan, A., Narayanan, S., Shiva, K., Ravi, I., Mayilvaganan, M., Pushpa, R., & Uma, S. (2021). Exploring differences in the physicochemical, functional, structural, and pasting properties of banana starches from dessert, cooking, and plantain cultivars (Musa spp.). *International Journal of Biological Macromolecules*, *191*, 1056–1067. https://doi.org/10.1016/j.ijbiomac.2021.09.172
18. Kumar, P. S., Saravanan, A., Sheeba, N., & Uma, S. (2019). Structural, functional characterization and physicochemical properties of green banana flour from dessert and plantain bananas (Musa spp.). *LWT*, *116*, 108524. https://doi.org/10.1016/j.lwt.2019.108524
19. Lim, J. (2011). Hedonic scaling: A review of methods and theory. *Food Quality and Preference*, https://doi.org/10.1016/j.foodqual.2011.05.008
20. Mora, M. R., Wang, Z., Goddard, J. M., & Dando, R. (2022). Consumers respond positively to the sensory, health, and sustainability benefits of the rare sugar allulose in yogurt formulations. *Foods*, *11*(22), 3718. https://doi.org/10.3390/foods11223718
21. Nelson, N. (1994). A photometric adaption of the Somogyi method for the determination of glucose. *J Biol Chem*, 153: 375–380.
22. Official methods of analysis. 1997. Gaithersburg, MD: Association of Official Analytical Chemists (AOAC) Volume II: Section 45.4.07, Method 985.29 (1997) 16th edn.
23. Official methods of analysis. 2005. Gaithersburg, MD: Association of Official Analytical Chemists (AOAC).18th edn.
24. Ovando-Martinez, M., Sáyago-Ayerdi, S., Agama-Acevedo, E., Goñi, I., & Bello-Pérez, L. A. (2008). Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chemistry*, 113(1), 121–126. https://doi.org/10.1016/j.foodchem.2008.07.035

25. Palframan, R., Gibson, G., & Rastall, R. (2003). Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. *Letters in Applied Microbiology*, 37(4), 281–284. https://doi.org/10.1046/j.1472-765x.2003.01398.x
26. Powthong, P., Jantrapanukorn, B., Suntornthiticharoen, P., & Laohaphatanalert, K. (2020). Study of prebiotic properties of selected banana species in Thailand. *Journal of Food Science and Technology*, 57(7), 2490–2500. https://doi.org/10.1007/s13197-020-04284-x
27. Ranganna, S. (1997) Handbook of Analysis and Quality Control for Fruit and Vegetable Products. Second Edition, Tata McGraw Hill Publishing Company Limited, New Delhi, 11-12.
28. Ravi, I., Mustaffa, M.M. (2013) Starch and amylose variability in banana cultivars. *Ind J Plant Physiol*. 18, 83–87. https://doi.org/10.1007/s40502-013-0014-2
29. Re, R., Pellegrini, N., Proteggente, A., Yang, M., & Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic Biol Med*, 26, 231-237. 17
30. Sadasivam., and Manickam. (2008). Biochemical Methods. 3rd edn, New Age International, New Delhi, pp 1-19.
31. Salvador, A., Sanz, T., & Fiszman, S. (2007). Changes in colour and texture and their relationship with eating quality during storage of two different dessert bananas. *Postharvest Biology and Technology*, *43*(3), 319–325. https://doi.org/10.1016/j.postharvbio.2006.10.007
32. Silva, C. R., Simoni, J. A., Collins, C. H., & Volpe, P. L. O. (1999). Ascorbic acid as a standard for iodometric titrations. An analytical experiment for general chemistry. *Journal of Chemical Education*, *76*(10), 1421. https://doi.org/10.1021/ed076p1421
33. Singleton, V.L., and Slinkard, K. (1977). Total phenol analysis: Automation and comparison with manual methods. *Am J Enol Vitic*, 2849-55.
34. Sorescu, A. A., Nuta, A., Ion, R. M., & Iancu, L. (2018). Qualitative analysis of phytochemicals from sea buckthorn and gooseberry. *Phytochemicals–Source of Antioxidants and Role in Disease Prevention*, 13, 161-77.
35. Vernaza, M. G., Gularte, M. A., & Chang, Y. K. (2011). Addition of green banana flour to instant noodles: rheological and technological properties. *Ciência E Agrotecnologia*, *35*(6), 1157–1165. https://doi.org/10.1590/s1413-70542011000600016
36. Von Loesecke, H. W. (1950). Bananas: chemistry, physiology, technology (No. 634.772/V947). Interscience Publishers.
37. Wani, I. A., Sogi, D. S., Wani, A. A., Gill, B. S., & Shivhare, U. S. (2010). Physico-chemical properties of starches from Indian kidney bean *(Phaseolus vulgaris*) cultivars. *International Journal of Food Science & Technology*, *45*(10), 2176–2185. https://doi.org/10.1111/j.1365-2621.2010.02379.x
38. Yadav, B. S., Yadav, R. B., Kumari, M., & Khatkar, B. S. (2014). Studies on suitability of wheat flour blends with sweet potato, colocasia and water chestnut flours for noodle making. *LWT-Food Science & Technology*, *57*(1), 352–358. https://doi.org/10.1016/j.lwt.2013.12.042

Websites

1. FAO 2014 Guidelines for assessing nutrition-related Knowledge, Attitudes and Practices https://www.fao.org/4/i3545e/i3545e00.htm
2. https://fssai.gov.in/cms/manuals-of-methods-of-analysis-for-various-food-products.php